



Protective nets reduce pollen flow in blueberry orchards

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ABSTRACT

Protective covers are commonly employed in agricultural systems to reduce the impacts of extreme weather events, pest species and to control the environmental conditions in which crop plants are grown. As protected cropping systems are expanding rapidly, there is an urgent need to better understand how variations in netting practices might impact pollination service delivery by wild and managed insects to pollinator dependent crops. We used southern highbush blueberry (*Vaccinium corymbosum* L. interspecific hybrid) crops to investigate (i) how variations in protected cropping structures (fully netted, partially netted and unnetted blocks) influence the amount and composition of pollen deposited on crop stigmas; (ii) to what extent blueberry floral abundance and plant richness in remnant vegetation influence pollen composition on crop stigmas; and (iii) the difference between stigmatic pollen load composition in the middle and at the edge of crop blocks. We collected data from 15 field blocks of 6 different cultivars distributed on 10 farms. We collected blueberry stigmas to analyse the pollen load and measured blueberry floral abundance and richness of flowering plant taxa in remnant vegetation every two weeks. Our results indicate that blueberry pollen abundance on stigmas was reduced by up to 81% under full netting and 36% by partial netting. On blueberry stigmas, we identified a total of 31 morphospecies of non-blueberry pollen from 20 plant families. There was no relationship between blueberry stigmatic pollen loads and blueberry floral abundance. Moreover, the composition of non-blueberry pollen on stigmas differed between blueberry blocks under different netting categories. However, there was no relationship between plant taxa present in the surrounding remnant vegetation of each block and the pollen load on the stigmas of each block. Combining all netting treatments, stigmas located at the edge of the blocks received a greater amount of both conspecific (5% more) and heterospecific (40% more) pollen grains than those within the middle of blocks. Pollen flow in fields is reduced under netting structures as well as in the middle of blocks. Reduced blueberry pollen flow under nets may be detrimental to fruit yield and quality for some varieties of pollinator dependent crops, particularly those that are self-incompatible.

1. Introduction

Protective nets are becoming increasingly common and are widely used over fields to protect them from damage caused by hailstorms, substantial rainfall, radiation and wind (Anderson et al., 2013; McCaskill et al., 2016a; Racsco and Schrader, 2012; Shahak et al., 2004). Nets can also be an alternative to reduce the cost of chemicals since they help to manage insect pests (Chouinard et al., 2016; Kuesel et al., 2019; Whitaker et al., 1999), as well as bird (Anderson et al., 2013) and bat consumption of fruit (Tollington et al., 2019). Despite the benefits that protective nets offer growers, studies have reported some adverse effects

on plant phenology (Solomakhin and Blanke, 2010; Stamps, 2009) and fruit quality (Leite et al., 2002) due to changes in temperature and humidity. Protective nets do not only affect plants, but can also affect managed insects introduced for pollination (Evans et al., 2019; Kendall et al., 2021; Pinzauti, 1994). However, we know little about the effects of protected cropping on pollination services to crops and how nets impact pollen flow between plants.

The impact of netting on pollinators and pollination services can vary with the type of net and the timing of netting deployment (Leech and Howe, 2014; Kendall et al., 2021). Further, the environmental conditions created under protective covers may be challenging for pollinators.

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Changes in wind direction, for instance, can disorient and reduce bee activity (Dag and Eisikowitch, 1995). High temperatures may cause a decrease in bee activity due to stress, and the intensity of UV light and humidity within enclosed fields can change bee foraging behaviour and impact their health (Abou-Shaara et al., 2017; Pinzauti, 1994). Yet few studies have actually compared covered and uncovered (control) environments when specifically studying impacts on pollination.

While some studies indicate that the control of solar radiation and temperature using netting can improve fruit physical appearance and quality (Chouinard et al., 2019; McCaskill et al., 2016b; Solomakhin and Blanke, 2010), anti-hail net can negatively impact the coloration of some fruits (Stroka et al., 2021), and plants growing under nets also have fewer buds and flowers, which consequently reduces the number of fruits (Snelgar et al., 1991). However, in apple and blueberry fields, netting can also result in delayed maturity (Chouinard et al., 2019; Lobos et al., 2009) and this feature can be used to facilitate both harvest and storage (Middleton and McWaters, 2002).

Protective nets can also restrict the movement and activity of pollinators, and consequently, may reduce pollination effectiveness (Foley et al., 2011), and ultimately pollinator health (Kendall et al., 2021). For example, the negative effects of enclosed environments include weakened colonies (da Silva et al., 2017), a decline in numbers of adult foragers, a lower number and shorter duration of foraging trips (Evans et al., 2019), and high susceptibility to stress and pathogen infestation (Morimoto et al., 2011).

As a wide variety of crops are grown in enclosure systems (Reddy, 2016) and most of these crops are fully or partially dependent on the presence of insect pollinators (Aizen et al., 2009), greater understanding of pollen flow in netted and un-netted environments is required. We used southern highbush blueberry (*Vaccinium corymbosum* L. interspecific hybrid) as a model system, to determine the extent to which pollen flow (conspicuous and heterospecific pollen) on stigmas varies in fully netted, partially netted and unnetted fields. Specifically, we ask (a) how do variations in protected cropping structures (fully netted, partially netted and unnetted blocks) influence pollen composition on stigmas? (b) To what extent does blueberry floral abundance and remnant vegetation richness influence pollen composition on stigmas? (c) Does stigmatic pollen load composition differ between the edge and middle of blueberry blocks? We use the outcomes of this research to inform recommendations for future management of blueberry pollination services under protected covers.

2. Methods

2.1. Study system

This study was conducted between the blueberry flowering season from March to July in 2020, using southern highbush blueberries, on 10 farms located between Macksville (30°41'56"S, 152°54'11"E) and Halfway Creek (29°56'20"S, 153°07'27"E), New South Wales, Australia. We focused on 6 different cultivars of southern highbush: 2 blocks of 'Snowchaser (Patent No.: USPP19503P3; AU PBR Grant No.: 4102)', 2 blocks of 'C99-42 (Patent No.: USPP20695P2; AU PBR Grant No.: 3570)', 5 blocks of 'Ridley 1111' (patented US20110185459P1; AU PBR Grant No.: 4089), 4 blocks of 'C00-09 (patented USPP22778P3; AU PBR Grant No.: 3662)', 1 block of 'OB1 (unpatented variety)', and 1 block of 'Ridley 4507 (AU PBR Grant No.: 6076)'. Most southern highbush cultivars vary in their mating system from partially self-incompatible to self-compatible (DeVetter et al., 2022; Taber and Olmstead, 2016), yet fruit size is generally improved with pollinator visits (Kendall et al., 2020; Lang and Danka, 1991). To determine the implications of changes in pollen flow, we tested the need for insect pollination of the cultivars included in the study comparing fruit set between flowers that were open to pollination (unmanipulated) and flowers that were kept bagged and excluded from pollinators (25 flowers used for each treatment in each cultivar).

Block size varied from 0.13 ~ 1.73 ha with an average of 0.63 ha. We sampled one blueberry block per farm, except for one farm which was large enough to sample 6 blocks at least 1 km apart. The landscape surrounding the blueberry farms was heterogeneous and all farms had patches of remnant vegetation scattered around berry blocks. The vegetation in the area is classified as sub-tropical and warm temperate rainforest, and wet sclerophyll forest (an Australian vegetation type characterised by the presence of plants like Eucalyptus, Acacias and Banksias associated with low soil fertility) (Sahukar et al., 2003).

We classified netting structures into three different treatments: fully netted (the whole block was enclosed with nets), partially netted (net on top with sides open) and unnetted (blocks without nets) (Fig. 1). The farms used bird netting with a mesh size of 10 ~ 23 mm and the height varied from 3 to 7 m with an average of 4 m. Blueberry bushes varied from 1.2 m to 1.8 m height.

The duration of net opening varied according to cultivar and time of year; For example, some blocks were unnetted for the entire flowering season and others were partially netted only for part of the flowering season (see details of netting management throughout the 13 weeks of experiment in Appendix A: Table S1). Netting management varied across a given farm as growers had blocks with different blueberry cultivars within their farms. On average, honey bee hive density across all the farms was 6 hives/ha, and farms varied in size, ranging from 4 ha to 1180 ha. Honey bee hives were placed between 40 and 140 m from the focal blueberry blocks.

2.2. Stigma sampling

We waited approximately 14 days after the first flower buds started opening to perform the first week of data collection as this is normally the time hives are deployed and at least 15% of the flowers were in bloom across all the blocks we sampled. Then, we collected blueberry stigmas at ongoing intervals of every 2 weeks, and each block was sampled 7 times (13 weeks of experiment in total). The last week of stigma sampling occurred at the end of flowering, when most of the flowers within the block had dropped off, and bee hives were removed.

In the first week, for each block, we randomly chose four rows and tagged 1 blueberry branch at the edge and 1 in the middle of each row, totalling 8 branches tagged per block and 120 in total. The distance between middle and edge varied from 3 to 78 m (21 ± 19 m on average) depending on block size. Every two weeks we collected one flower from the same tagged branch and the stigma was mounted on a slide with Fuchsin gelatine (Dafni, 1992) immediately after collection. Flowers buds were tagged at an early stage of development, taking up to 4–5 days to open. To make sure that pollinators had time to deposit pollen on the stigmas before the stigmas were no longer receptive, we waited at least 48 h from the day the flowers opened until stigma collection. The pollen grains present on the blueberry stigmas were quantified in the laboratory, and we counted every pollen grain found on the slide and classified them as either conspecific (blueberry) or heterospecific (non-blueberry). We identified the heterospecific pollen to family level using our pollen reference collection, the Australasian Pollen and Spore Atlas (APSA), and existing literature (Erdtman, 1986; Moore and Webb, 1978; Salgado-Labouriau, 1973).

From the 15 blocks in which we performed our experiments, we were only able to collect harvest data for 11 blocks. However, parts of these blocks were affected by blueberry rust (a plant disease caused by the fungus *Thekopsora minima*) after the flowering season in the 2020–21 season, so they produced much less fruit than expected. All farms collected harvest data in different ways hence we could not compare harvest data across blocks as we could not standardize methods and measures; For example, some growers estimated the total amount of fruit on their property (which includes different cultivars and netting conditions) rather than per block; others provided tray level estimates that comprised multiple blocks. Therefore, we were not able to statistically compare the fruit set between different netting treatments.



Fig. 1. a) unnetted, b) partially netted, and c) fully netted blueberry blocks.

2.3. Flower abundance surveys

Blueberry floral abundance was measured on the same day that stigma sampling occurred. As the primary question was about how floral abundance impacts pollen loads on stigmas, we did not collect early or late data on flowering - before and after bee hives were deployed - as it would not be relevant to stigma collection. Counts of open flowers were conducted on 10 tagged blueberry plants in each block (5 plants at the edge and 5 in the middle). From 4–49 m (14 ± 12 m) adjacent to all the blocks, there were scattered patches of remnant and regrowth non-crop native vegetation as well as weeds. These patches varied from 0.18 to 1.84 ha (0.58 ± 0.47 ha) in size. We established two 20 m long vegetation transects in the nearest patch to the focal block and performed floral abundance and richness surveys every week of data collection. One transect was established along the edge and the other from the edge into the middle of the patch. We recorded the plants and number of flowers every two weeks. We also collected the anthers of all the flowering plants present in the area weekly and mounted them on slides for a pollen reference collection.

2.4. Statistical analysis

The statistical tests described below were performed in R environment (R Core Team, 2022), and all the GLMM models were performed using the 'glmmTMB' package (Brooks et al., 2017) and checked for uniformity and dispersion using DHARMA package (Hartig and Hartig, 2017).

2.4.1. Variations in protected cropping structures (fully netted, partially netted and unnetted blocks), blueberry floral abundance and pollen composition on stigmas

We compared the number of conspecific and heterospecific pollen grains on blueberry stigmas to test if they differed among fully netted ($n = 512$), partially netted ($n = 144$), and unnetted blocks ($n = 184$) and if blueberry floral abundance would influence the composition. We fitted two generalized linear mixed-effects models (GLMMs): one with the number of conspecific pollen grains as response variable and a negative binomial distribution, and the second model with heterospecific pollen grains as the response variable and with zero-inflated negative binomial distribution. While the negative binomial distribution allowed us to account for overdispersion in the data, the zero-inflation component allowed us to account for the large number of zero count observations. The treatment (blocks fully netted, partially netted, or unnetted) and the abundance of blueberry flowers were considered fixed effects. The size of the net mesh was also included as an interaction with treatment to evaluate if there was variation in pollen deposition within each netting category as a result of mesh size. Plant nested within block, week, and cultivar were included as random effects in both models. Statistical significance was tested using likelihood ratio tests comparing the model with and without the fixed factors (i.e., null model) using Anova function in the car package (Fox and Weisberg, 2018). We also performed a post hoc test comparing the number of

pollen grains among each treatment using emmeans package (Searle et al., 1980) and determined significance using P values (significance was taken as $P < 0.05$).

In addition, to analyse if the quantity of conspecific and heterospecific pollen grains changed with respect to netting conditions in the middle of the flowering season, we grouped only the blocks that changed from partially netted to fully netted ($n = 224$ observations from 4 blocks) and the blocks that changed from unnetted to fully netted ($n = 168$ observations from 3 blocks). We then applied GLMMs with the period (before and after netting change) as the fixed effect and the same random effects included in the previous models. We used negative binomial distribution in the conspecific pollen models and zero-inflation negative binomial distribution in the heterospecific pollen models.

2.4.2. Plant taxonomic richness in remnant vegetation and pollen composition on blueberry stigmas

To test differences in pollen composition among the different netting treatments, we first verified the multivariate homogeneity of group variance (function betadisper; package vegan; Oksanen et al., 2022). Then we performed a permutational multivariate analysis of variance (PERMANOVA) (function adonis; package vegan; Oksanen et al., 2022) using Bray-Curtis distance and 999 permutations. The similarities and dissimilarities in pollen composition among the different netting treatments were visually assessed using non-metric, multidimensional scaling ordinations (nMDS) with two dimensions. Lastly, we performed an indicator species analysis using the indval function from the indicspecies package (Cáceres and Legendre, 2009) to identify the pollen types that were associated to each netting treatment.

To test if there was a relationship between the plant taxa present in the remnant vegetation and on the blueberry stigmas, we used Procrustes analysis which allow us to compare the two original data tables (i.e., plant taxa in the remnant vegetation and pollen morphospecies on blueberry stigmas). We built two matrices, one for the remnant vegetation and one for the blueberry stigmas. Plants in the remnant vegetation and pollen types found on the stigmas received compatible identification (morphospecies/morphotypes within each plant taxon). We first performed a Principal Component Analysis (PCA) based on Euclidean distances, then we used the function procrustes from vegan package (Oksanen, 2015) to run the analysis. To evaluate the significance (non-randomness) of the observed m^2 statistic we used the Protest permutation test (protest function; 1000 permutations; (Oksanen, 2015).

2.4.3. Stigmatic pollen load composition at the edge and in the middle of blueberry block

To compare the pollen load (conspecific and heterospecific pollen grains) deposited by pollinators on blueberry stigmas located at the edge and in the middle of the fully netted, partially netted, and unnetted blocks we applied GLMMs with the location of the stigma within the block as the fixed effect and the pollen load as the response variable. Distance between middle and edge nested within block, plant nested within block, week, and cultivar were included as random effects in the models. We again used negative binomial distribution in the conspecific

pollen models and zero-inflation negative binomial distribution in the heterospecific pollen models.

3. Results

3.1. How do variations in protected cropping structures (fully netted, partially netted and unnetted blocks) influence pollen composition on stigmas?

We counted a total of 41,779 pollen grains on 840 blueberry stigmas and the amount of conspecific pollen on stigmas was affected by the netting type ($X^2 = 362.3$, $df = 2$, $P < 0.001$, Table 1). The average \pm SD number of pollen grains on stigmas of unnetted blocks was 96.67 ± 49 (and 49% of the total amount of conspecific pollen grains counted), 58.88 ± 48 (and 25% of the total amount) in partially netted, and 18.30 ± 45 (and 24% of the total amount) in fully netted blocks. Partially and fully netted blocks received less pollen comparing to unnetted blocks (*emmeans* unnetted - partially netted: t -value = 4.16, $P < 0.001$; *emmeans* unnetted - fully netted: t -value = -12.47, $P < 0.001$), and fully netted blocks also received significantly less pollen grains than partially netted blocks (*emmeans*: t -value = -10.94, $P < 0.001$) (Fig. 2). All cultivars were deemed to benefit from insect pollination as our trials revealed that heavier fruits were produced in flowers open to pollination (mean $2.34 \text{ g} \pm \text{SD } 0.77 \text{ g}$) compared to flowers that were kept bagged and excluded from pollination (mean $1.25 \text{ g} \pm \text{SD } 0.79 \text{ g}$).

We identified a total of 31 pollen morphospecies distributed in 20 families present on blueberry stigmas (Fig. S1), and netting had a significant effect on heterospecific pollen deposition as well ($X^2 = 70.5$, $df = 2$, $P < 0.001$, Table 1). Of the stigmas with heterospecific pollen, partially and fully netted blocks received less heterospecific pollen grains per stigma than unnetted blocks (*emmeans* unnetted - partially netted: t -value = 4.191, $P < 0.001$; *emmeans* unnetted - fully netted: t -value = -11.94, $P < 0.001$), and partially netted received less heterospecific pollen grains than fully netted blocks (*emmeans*: t -value = -6.86, $P < 0.001$). Unnetted blocks had an average of 17.85 ± 16 heterospecific pollen grains on the stigmas (and 57% of the total amount of heterospecific pollen grains counted), partially netted 9.55 ± 14 (and 25% of the total amount), and fully netted blocks had an average of only 2 ± 13 (and 17% of the total amount) (Fig. 2). While the netting treatments affected pollen deposition (both conspecific and heterospecific

pollen), there was no significant correlation between pollen grain abundance and the size of the net mesh used (conspecific pollen: $X^2 = 2.22$, $df = 1$, $P = 0.135$; heterospecific pollen: $X^2 = 2.45$, $df = 1$, $P = 0.117$).

Moreover, for blocks in which the netting condition changed over time, there was a significant reduction in both heterospecific and conspecific pollen grains after the change from partially netted to fully netted ($X^2 = 43.49$, $df = 1$, $P < 0.001$; $X^2 = 60.2$, $df = 1$, $P < 0.001$, respectively), and from unnetted to fully netted (heterospecific: $X^2 = 27.57$, $df = 1$, $P < 0.001$; conspecific: $X^2 = 23.04$, $df = 1$, $P < 0.001$).

The raw data of fruit production per block can be found in Table S2.

3.2. To what extent does blueberry floral abundance and plant taxa in remnant vegetation influence pollen composition on stigmas?

The amount of both conspecific and heterospecific pollen grains on the stigmas was not related to the number of open blueberry flowers in the blocks ($X^2 = 3.3$, $df = 1$, $P = 0.069$; $X^2 = 1.57$, $df = 1$, $P = 0.209$, respectively). Flowers of the cultivars studied here were present throughout the blooming period and did not present a clear peak bloom period during the study (Fig. 4).

There was no significant variation in dispersion comparing the three different netting groups ($F = 0.59$; $P = 0.54$; 999 permutations), which implies that PERMANOVA was unaffected by heterogeneity (Anderson and Walsh, 2013). Results of PERMANOVA showed that pollen morphospecies composition on the stigmas was different under different netting treatments (unnetted, partially netted, and fully netted) ($r^2 = 0.13$, $P < 0.001$, Fig. 5). Pollen morphospecies associated with each treatment can be found in Table S3. However, even though blocks under different netting categories had different pollen loads, this difference could not be explained by the plant taxa recorded in the remnant vegetation around the blocks, demonstrated by a lack of significant correlation between the two data sets (Procrustes $r = 0.10$, $m^2 = 0.98$, $P = 0.578$).

3.3. Does stigmatic pollen load composition differ between the edge and middle of blueberry blocks?

Overall, stigmas located at the edge of the blocks received a greater amount of both conspecific and heterospecific pollen grains than stigmas in the middle of blocks. In unnetted blocks, the average \pm SD number of conspecific pollen grains on the stigmas located at the edge was 103 ± 42 grains and in the middle was 89 ± 40 ($X^2 = 4.71$, $df = 1$, $P = 0.02$, Fig. 3a). The same pattern was found for heterospecific pollen in these blocks, where the average amount of pollen grains in the middle (11 ± 15) and at the edge (24 ± 21) was significantly different ($X^2 = 12.12$, $df = 1$, $P < 0.001$, Fig. 3a). With an average of 64 ± 49 pollen grains in the middle and 59 ± 46 at the edge, partially netted blocks did not differ in the amount of conspecific pollen ($X^2 = 0.27$, $df = 1$, $P = 0.59$, Fig. 3b). However, a significant greater amount of heterospecific pollen was found at the edge of these blocks ($X^2 = 14.27$, $df = 1$, $P < 0.001$, Fig. 3b), presenting an average of 14 ± 17 grains at the edge, and only 5 ± 7 in the middle. Lastly, the lowest amounts of pollen were found in fully protected blocks, but only heterospecific pollen was significantly different between middle and edge. We found an average of 1 ± 3 heterospecific pollen in the middle and 3 ± 4 at the edge ($X^2 = 14.9$, $df = 1$, $P < 0.001$, Fig. 3c), and 17 ± 17 conspecific pollen grains in the middle and 19 ± 20 at the edge ($X^2 = 1.90$, $df = 1$, $P = 0.16$, Fig. 3c).

4. Discussion

The results of this study indicate that protective nets affect pollen flow in blueberry fields by reducing pollen deposition on the stigmas. The number of conspecific pollen was lower on stigmas under fully netted blocks compared to partially netted and unnetted blocks. Although the use of bird netting is important in blueberry fields

Table 1

Results of Generalized Linear Mixed Models evaluating the effects of the netting treatment and blueberry flower abundance on pollen composition on blueberry stigmas. Estimate (fixed factors) or variance (random factors), standard error (fixed factors) or standard deviation (random factors), z and P values are shown.

Conspecific pollen				
Variables	Estimate/ variance	SE/SD	z	P
Intercept	2.8459312	0.1933540	14.719	< 0.001
Netting - unnetted	1.6098892	0.0932742	17.260	< 0.001
Netting - partially netted	0.9717777	0.0888906	10.932	< 0.001
Flower abundance	0.0013705	0.0007542	1.817	0.0692
Net mesh size	0.0005694	0.0276642	0.021	0.9835
Week (random)	0.0004301	0.02074		
Variety (random)	0.1241914	0.35241		
Plant/block (random)	0.0823426	0.28695		
Heterospecific pollen				
Variables	Estimate/ variance	SE/SD	z	P
Intercept	2.974630	0.229057	12.986	< 0.001
Netting - partially netted	-0.482314	0.163417	-2.951	0.00316
Netting - fully netted	-1.200847	0.151029	-7.951	< 0.001
Flower abundance	0.001902	0.001515	1.255	0.20938
Net mesh size	-0.021078	0.030482	-0.691	0.48926
Week (random)	5.611e-12	2.369e-06		
Variety (random)	4.598e-02	2.144e-01		
Plant/block (random)	9.634e-02	3.104e-05		

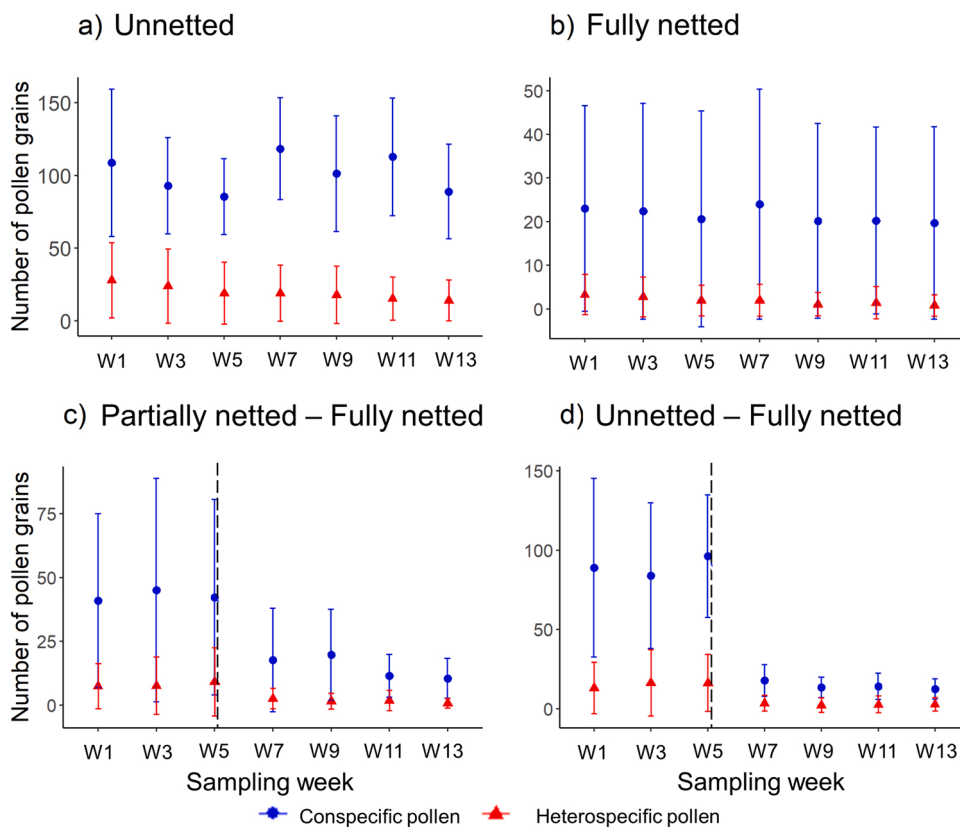


Fig. 2. Number of conspecific (blue) and heterospecific (red) pollen grains observed on the stigmas throughout the blueberry flowering season in (a) unnetted (2 blocks), (b) fully netted (5 blocks), (c) partially netted changed to fully netted (4 blocks) and (d) unnetted changed to fully netted blocks (3 blocks) in each of the 7 sampling rounds. Dashed black lines indicate when the netting was changed.

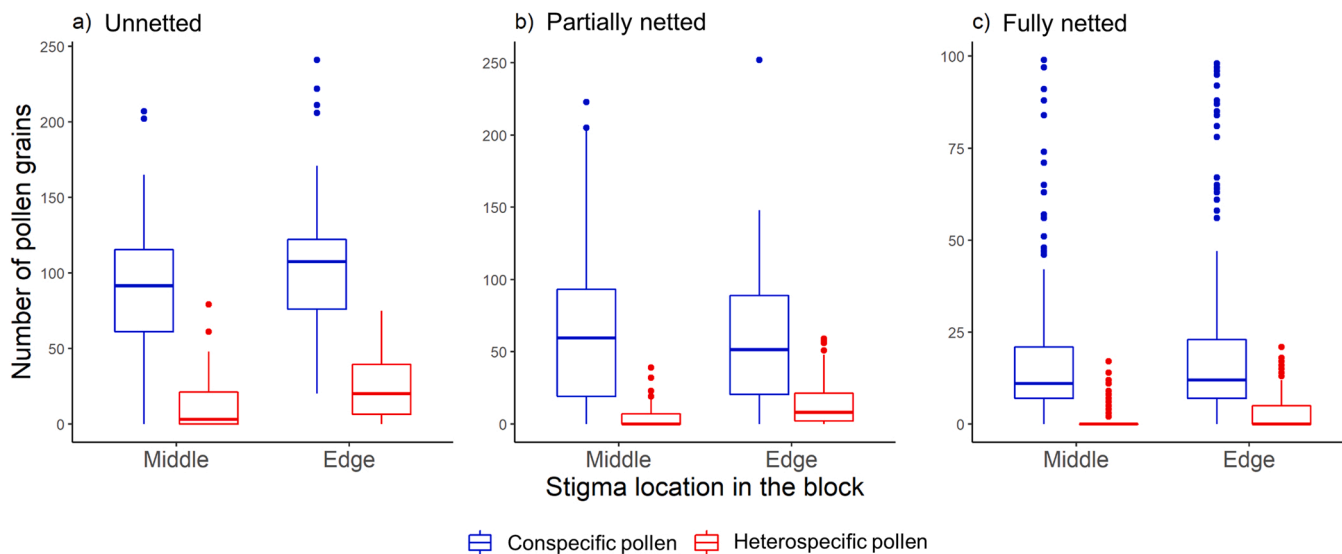


Fig. 3. Amount of conspecific (green) and heterospecific (red) pollen grains on stigmas in the middle and at the edge of a) unnetted, b) partially netted, and c) fully netted blocks.

(Goodman and Clayton-Greene, 1988) and increasingly becoming popular in other high-value crops (Reddy, 2016), our results indicate that the timing of net installation clearly needs to be considered. Some studies report better insect pollination when nets were applied only after full bloom (do Amarante et al., 2011; Kiprijanovski et al., 2016). We observed that the blueberry cultivars in this study have a variable and long flowering period. Consequently, the timing of when to install or

remove protective nets can be challenging, especially for growers growing more than one cultivar in the same block. For example, in the two-week time interval when the netting was erected in some blocks, a significant reduction in the amount of pollen deposited on stigmas was evident. This confirms that pollinator movement in and out of the blocks is restricted when fields are fully netted (Evans et al., 2019; Pinzauti, 1994). However, less pollen flow is not necessarily a negative impact

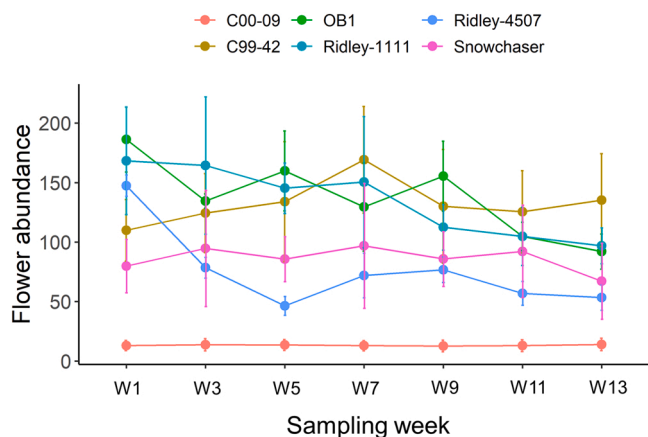


Fig. 4. Blueberry cultivars presented different flower abundance for each of the sampling rounds with no clear peak bloom throughout the study period.

depending on the plant-mating system of the cultivars grown. All the southern highbush cultivars studied here exhibited some level of self-compatibility and therefore are less dependent on pollinators, however, like Kendall et al. (2020), we found that flowers open and available for pollinator visits resulted in heavier fruits for most cultivars. Despite the differences in pollination needs, all blueberry cultivars benefit from pollinator visits to increase the stigmatic pollen loads in the case of self-compatible cultivars (Dogterom et al., 2000), or to promote cross-pollination in self-incompatible cultivars (Payne et al., 1988). Therefore, netting structure should be considered when the presence of pollinators is required.

The amount of heterospecific pollen on blueberry stigmas was always less than the amount of conspecific pollen grains. Further, the amounts of heterospecific pollen grains were even lower on stigmas in the middle of the blocks. Previous work in this system indicated that visitation on edges of protected tunnels resulted in higher berry weight and average yield per plant (Hall et al., 2020). Here, we found that flowers at the edge had both more conspecific as well as heterospecific pollen on the stigmas, but how this affects fruit production is unclear

(Fernández et al., 2012). Plants in open, unnetted blocks, or located at the edge of the partially netted and fully netted blocks are likely easier for pollinators to access from the remnant vegetation, where different insects such as native bees might be present (Nel et al., 2017; Nielsen et al., 2017), and probably offer easily accessible floral rewards for visitors that cannot fly through the nets, such as some birds and larger insects like moths. Moreover, edges are more moderate environments for pollinator foraging when compared to centre of tunnels due to the impact of protective tunnels on microclimate conditions (Hall et al., 2020). However, this has not been clearly demonstrated for protective netting (especially bird netting).

While pollen loads differed among the netting treatments, the Procrustes analysis showed that the pollen morphospecies found on the stigmas within each block were not related to the plant taxa present in the remnant vegetation near each block. This may suggest that flower visitors are not only visiting the local plant taxa next to the block but may be (i) selectively foraging on particular resources found far away from the block, (ii) have differing capabilities with respect to flying in and out of the blocks to access the floral resources, resulting in different pollen loads on the stigmas, and/or (iii) foraging on weeds (e.g., white clover) that may present in the inter-rows and not present in the remnant vegetation. Even though the dominant flower visitors, honey bees, generally exhibit floral constancy (Free, 1963), they tend to choose abundant plant species with a greater number of flowers in the area (Grüter et al., 2011; Magrach et al., 2017). In addition, even though honey bees can communicate the location of resources to their hive mates (Seeley and Visscher, 1988), the difference in the composition of the stigmatic pollen load between blocks with different netting may also be related to the number of flower visitors in unnetted blocks being able to more freely move in and out of the blocks to access certain resources as nets can present a significant barrier to pollinator movement (Kendall et al., 2021).

Although heterospecific pollen was deposited on stigmas, the amount of conspecific pollen was considerably higher. Other studies have shown that alternative floral resources can enhance pollination success in agroecosystems (Eckert et al., 2022; Hardman et al., 2016) as opposed to competition effects. Perhaps, strategies to facilitate pollinator access to these resources could result in better pollination and yield, but we still need to better understand the interactions between

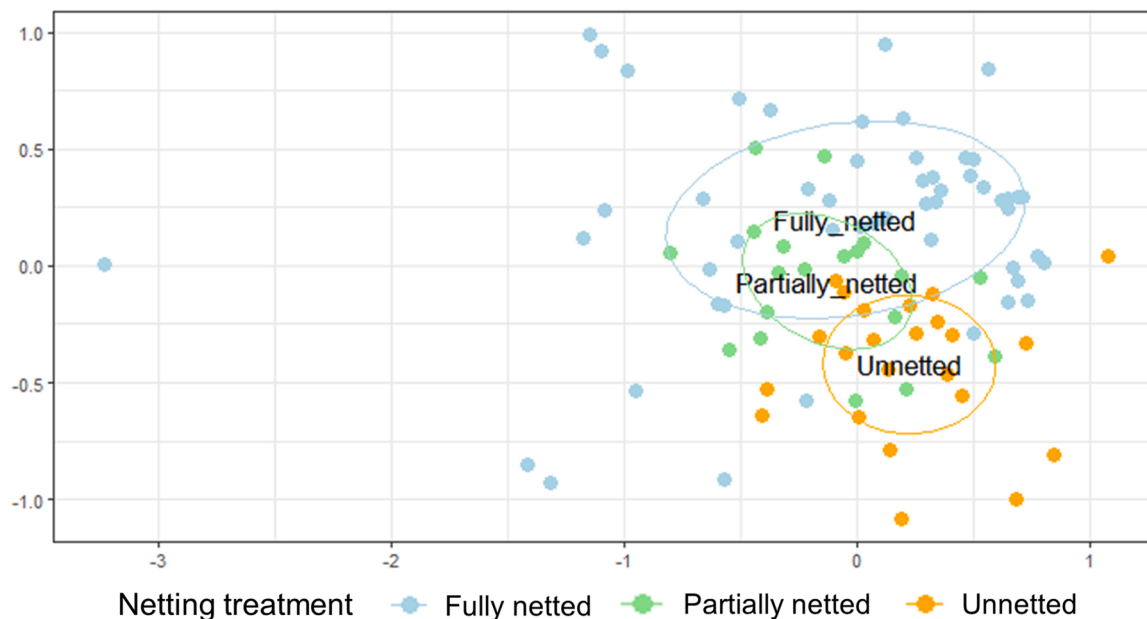


Fig. 5. Results of nMDS analysis for the abundance of pollen morphospecies on blueberry stigmas across the three different net categories (stress = 0.1647). Each point represents a unique weekly survey within each treatment. Net treatments are labelled on the right-hand side and coloured accordingly. Ellipses are showing standard deviation obtained from the variability measures around clusters calculated with nMDS.

crop and non-crop floral resources (Jones and Rader, 2022).

We did not find a relationship between mesh size and the amount of pollen deposited onto the stigmas. Mesh sizes greater than 12 mm are normally recommended to avoid limitations on pollinator movement (Rigden, 2008) and, in our study, most of the blocks had nets with mesh sizes greater than that. However, depending on the size of the pollinator's body, age, and experience flying through nets, pollinators could still have pollen pellets and grains removed by crawling through the nets. Hence, more evidence is needed to explain the mechanisms behind the reduction in pollen loads on stigmas under protective netting.

This study provides novel insight into how protective cropping practices influence pollen flow, but there are still important research questions to answer about trade-offs between the economic costs of netting practices in terms of lost pollination services and the benefits nets provide in terms of reducing bird damage to fruit. Future research efforts are needed to evaluate differences in fruit yield and quality in netted versus unnetted and edge versus the middle of blueberry fields to better understand the impact of protective nets on pollination services.

5. Conclusions

The timing and management of the deployment of netting structures is necessary to maximise pollen flow in fully netted crops that rely on pollinators to produce marketable fruits. Here, we found a significant decrease in the pollen flow to stigmas under netting. As stigmas located in unnetted blocks or at the edge of the blocks had higher average amounts of both hetero- and conspecific pollen grains, it is likely that pollinators are limited in their access to foraging resources in enclosed environments. From the three netting treatments studied here (unnetted, partially netted and fully netted), unnetted blocks were more conducive to the movement of conspecific pollen grains and therefore, will likely result in higher fruit set and quality. However, as protective netting structures are important for bird control, our results indicate that shifting from fully to partially netted structures would benefit blueberry production by potentially increasing the amount of conspecific pollen deposited on the stigmas.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2023.108544](https://doi.org/10.1016/j.agee.2023.108544).

References

- Abou-Shaara, H.F., Owayss, A.A., Ibrahim, Y.Y., Basuny, N.K., 2017. A review of impacts of temperature and relative humidity on various activities of honey bees. *Insect Soc.* 64, 455–463. <https://doi.org/10.1007/s00040-017-0573-8>.
- Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., Klein, A.M., 2009. How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. *Ann. Bot.* 103, 1579–1588.
- do Amarante, C.V.T., Steffens, C.A., Argenta, L.C., 2011. Yield and fruit quality of 'Gala' and 'Fuji' apple trees protected by white anti-hail net. *Sci. Hortic.* 129, 79–85. <https://doi.org/10.1016/j.scienta.2011.03.010>.
- Anderson, A., Lindell, C.A., Moxcey, K.M., Siemer, W.F., Linz, G.M., Curtis, P.D., Carroll, J.E., Burrows, C.L., Boulanger, J.R., Steensma, K.M.M., Shwiff, S.A., 2013. Bird damage to select fruit crops: The cost of damage and the benefits of control in five states. *Crop Prot.* 52, 103–109. <https://doi.org/10.1016/j.cropro.2013.05.019>.
- Anderson, M.J., Walsh, D.C., 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: what null hypothesis are you testing? *Ecol. Monogr.* 83, 557–574.
- Brooks, M.E., Kristensen, K., Van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Machler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. <https://doi.org/10.3929/ETHZ-B-000240890>.
- Cáceres, M.D., Legendre, P., 2009. Associations between species and groups of sites: indices and statistical inference. *Ecology* 90, 3566–3574. <https://doi.org/10.1890/08-1823.1>.
- Chouinard, G., Firlej, A., Cormier, D., 2016. Going beyond sprays and killing agents: Exclusion, sterilization and disruption for insect pest control in pome and stone fruit orchards. *Sci. Hortic.* 208, 13–27. <https://doi.org/10.1016/j.scienta.2016.03.014>.
- Chouinard, G., Veilleux, J., Pelletier, F., Larose, M., Phillion, V., Joubert, V., Cormier, D., 2019. Impact of Exclusion Netting Row Covers on 'Honeycrisp' Apple Trees Grown under Northeastern North American Conditions: Effects on Photosynthesis and Fruit Quality. *Insects* 10, 214. <https://doi.org/10.3390/insects10070214>.
- Dafni, A., 1992. *Pollination Ecology: A Practical Approach*. Oxford University Press.
- Dag, A., Eisikowitch, D., 1995. The influence of hive location on honeybee foraging activity and fruit set in melons grown in plastic greenhouses. *Apidologie* 26, 511–519.
- DeVetter, L.W., Chabert, S., Milbrath, M.O., Mallinger, R.E., Walters, J., Isaacs, R., Galinato, S.P., Kogan, C., Brouwer, K., Melathopoulos, A., Eeraerts, M., 2022. Toward evidence-based decision support systems to optimize pollination and yields in highbush blueberry. *Front. Sustain. Food Syst.* 6, 1006201. <https://doi.org/10.3389/fsufs.2022.1006201>.
- Dogterom, M.H., Winston, M.L., Mukai, A., 2000. Effect of pollen load size and source (self, outcross) on seed and fruit production in highbush blueberry cv. 'Bluecrop' (*Vaccinium corymbosum*; Ericaceae). *Am. J. Bot.* 87, 1584–1591. <https://doi.org/10.2307/2656734>.
- Eckert, P.W., Albrecht, M., Bertrand, C., Gobet, E., Herzog, F., Pfister, S.C., Tinner, W., Entling, M.H., 2022. Effects of temporal floral resource availability and non-crop habitats on broad bean pollination. *Land. Ecol.* 37, 1573–1586. <https://doi.org/10.1007/s10980-022-01448-2>.
- Erdtman, G., 1986. *Pollen morphology and plant taxonomy: angiosperms*. Brill Archive.
- Evans, L.J., Cutting, B.T., Jochym, M., Janke, M.A., Felman, C., Cross, S., Jacob, M., Goodwin, M., 2019. Netted crop covers reduce honeybee foraging activity and colony strength in a mass flowering crop. *Ecol. Evol.* 9, 5708–5719. <https://doi.org/10.1002/ece3.5154>.
- Fernández, J.D., Bosch, J., Nieto-Ariza, B., Gómez, J.M., 2012. Pollen limitation in a narrow endemic plant: geographical variation and driving factors. *Oecologia* 170, 421–431. <https://doi.org/10.1007/s00442-012-2312-1>.
- Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., Siebert, S., Tilman, D., Zaks, D.P.M., 2011. Solutions for a cultivated planet. *Nature* 478, 337–342. <https://doi.org/10.1038/nature10452>.
- Fox, J., Weisberg, S., 2018. *An R Companion to Applied Regression*. Sage publications.
- Free, J.B., 1963. The flower constancy of honeybees. *J. Anim. Ecol.* 32, 119. <https://doi.org/10.2307/2521>.
- Goodman, R., Clayton-Greene, K., 1988. Honeybee pollination of highbush blueberries (*Vaccinium corymbosum*). *Aust. J. Exp. Agric.* 28, 287. <https://doi.org/10.1071/EA9880287>.
- Grüter, C., Moore, H., Firmin, N., Helanterä, H., Ratnieks, F.L.W., 2011. Flower constancy in honey bee workers (*Apis mellifera*) depends on ecologically realistic rewards. *J. Exp. Biol.* 214, 1397–1402. <https://doi.org/10.1242/jeb.050583>.
- Hall, M.A., Jones, J., Rocchetti, M., Wright, D., Rader, R., 2020. Bee Visitation and Fruit Quality in Berries Under Protected Cropping Vary Along the Length of Polytunnels. *J. Econ. Entomol.* 113, 1337–1346. <https://doi.org/10.1093/jee/toaa037>.
- Hardman, C.J., Norris, K., Nevard, T.D., Hughes, B., Potts, S.G., 2016. Delivery of floral resources and pollination services on farmland under three different wildlife-friendly schemes. *Agric., Ecosyst. Environ.* 220, 142–151. <https://doi.org/10.1016/j.agee.2016.01.015>.
- Hartig, F., Hartig, M.F., 2017. Package 'DHARMA'.
- Jones, J., Rader, R., 2022. Pollinator nutrition and its role in merging the dual objectives of pollinator health and optimal crop production. *Philos. Trans. R. Soc. B* 377, 20210170. <https://doi.org/10.1098/rstb.2021.0170>.
- Kendall, L.K., Gagic, V., Evans, L.J., Cutting, B.T., Scalzo, J., Hanusch, Y., Jones, J., Rocchetti, M., Sonter, C., Keir, M., 2020. Self-compatible blueberry cultivars require fewer floral visits to maximize fruit production than a partially self-incompatible cultivar. *J. Appl. Ecol.* 57, 2454–2462.

- Kendall, L.K., Evans, L.J., Gee, M., Smith, T.J., Gagic, V., Lobaton, J.D., Hall, M.A., Jones, J., Kirkland, L., Saunders, M.E., 2021. The effect of protective covers on pollinator health and pollination service delivery. *Agric., Ecosyst. Environ.* 319, 107556.
- Kiprijanovski, M., Gjamovski, V., Arsov, T., 2016. The effects of anti-hail net in protection of pear orchard after hailstorm occurrence. *Acta Hort.* 529–534. <https://doi.org/10.17660/ActaHortic.2016.1139.91>.
- Kuesel, R., Scott Hicks, D., Archer, K., Sciligo, A., Bessin, R., Gonthier, D., 2019. Effects of Fine-Mesh Exclusion Netting on Pests of Blackberry. *Insects* 10, 249. <https://doi.org/10.3390/insects10080249>.
- Lang, G.A., Danka, R.G., 1991. Honey-bee-mediated Cross- versus Self-pollination of 'Sharpblue' Blueberry Increases Fruit Size and Hastens Ripening. *JASHS* 116, 770–773. <https://doi.org/10.21273/JASHS.116.5.770>.
- Leech, M., Howe, T., 2014. Understanding practices in key pollination areas. Sydney, NSW: Hort. Aust. Ltd 57.
- Leite, G.B., Petri, J.L., Mondardo, M., 2002. Efeito da tela antigranizo em algumas características dos frutos de macieira. *Rev. Bras. Frutic.* 24, 714–716. <https://doi.org/10.1590/S0100-29452002000300037>.
- Lobos, G.A., Retamales, J.B., del Pozo, A., Hancock, J.F., Flore, J.A., 2009. PHYSIOLOGICAL RESPONSE OF *Vaccinium corymbosum* "ELLIOTT" TO SHADING NETS IN MICHIGAN. *Acta Hort.* 465–470. <https://doi.org/10.17660/ActaHortic.2009.810.60>.
- Magrath, A., González-Varo, J.P., Boiffier, M., Vilà, M., Bartomeus, I., 2017. Honeybee spillover reshuffles pollinator diets and affects plant reproductive success. *Nat. Ecol. Evol.* 1, 1299–1307. <https://doi.org/10.1038/s41559-017-0249-9>.
- McCaskill, M.R., McClymont, L., Goodwin, I., Green, S., Partington, D.L., 2016a. How hail netting reduces apple fruit surface temperature: A microclimate and modelling study. *Agric. For. Meteorol.* 226–227, 148–160. <https://doi.org/10.1016/j.agrformet.2016.05.017>.
- McCaskill, M.R., McClymont, L., Goodwin, I., Green, S., Partington, D.L., 2016b. How hail netting reduces apple fruit surface temperature: A microclimate and modelling study. *Agric. For. Meteorol.* 226–227, 148–160. <https://doi.org/10.1016/j.agrformet.2016.05.017>.
- Middleton, S., McWaters, A., 2002. Hail netting of apple orchards—Australian experience. *Compact Fruit. Tree* 35, 51–55.
- Moore, P.D., Webb, J.A., 1978. An illustrated guide to pollen analysis.
- Morimoto, T., Kojima, Y., Toki, T., Komeda, Y., Yoshiyama, M., Kimura, K., Nirasawa, K., Kadowaki, T., 2011. The habitat disruption induces immune-suppression and oxidative stress in honey bees: Habitat Disruption of honey bees. *Ecol. Evol.* 1, 201–217. <https://doi.org/10.1002/ece3.21>.
- Nel, L., Pryke, J.S., Carvalheiro, L.G., Thébault, E., van Veen, F.J.F., Seymour, C.L., 2017. Exotic plants growing in crop field margins provide little support to mango crop flower visitors. *Agric., Ecosyst. Environ.* 250, 72–80. <https://doi.org/10.1016/j.agee.2017.09.002>.
- Oksanen, J., 2015. *Vegan: an introduction to ordination*.
- Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Durand, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Devand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lathi, L., McGlenn, D., Ouellette, M.-H., Cunha, E.R., Smith, T., Stier, A., Ter Braak, C., Weedon, J., 2022. *Community Ecology Package*.
- Payne, J.A., Amis, A.A., Cane, J.H., Lyrene, P.M., 1988. Fruit size, seed size, seed viability and pollination of rabbiteye blueberries (*Vaccinium ashei* Reade), in: IV International Symposium on *Vaccinium Culture* 241. pp. 38–43.
- Pinzauti, M., 1994. Pollinator behaviour and activity in colonies of *Apis mellifera* in confined environments. *Ethol. Ecol. Evol.* 6, 101–106. <https://doi.org/10.1080/03949370.1994.10721981>.
- Racsko, J., Schrader, L.E., 2012. Sunburn of Apple Fruit: Historical Background, Recent Advances and Future Perspectives. *Crit. Rev. Plant Sci.* 31, 455–504. <https://doi.org/10.1080/07352689.2012.696453>.
- Reddy, P.P., 2016. *Sustainable Crop Protection Under Protected Cultivation*. Springer,.
- Rigden, P., 2008. To net or not to net, 3rd ed. The State of Queensland, Department of Primary Industries and Fisheries.
- Sahukar, R., Gallery, C., Smart, J., Mitchell, P., 2003. The bioregions of New South Wales: their biodiversity, conservation and history. National Parks and Wildlife Service, Hurstville NSW.
- Salgado-Labouriau, M.L., 1973. Contribuição à palinologia dos cerrados. *Academia Brasileira de Ciências Rio de Janeiro*.
- Searle, S.R., Speed, F.M., Milliken, G.A., 1980. Population Marginal Means in the Linear Model: An Alternative to Least Squares Means. *Am. Stat.* 34, 216–221. <https://doi.org/10.1080/00031305.1980.10483031>.
- Seeley, T.D., Visscher, P.K., 1988. Assessing the benefits of cooperation in honeybee foraging: search costs, forage quality, and competitive ability. *Behav. Ecol. Sociobiol.* 22, 229–237.
- Shahak, Y., Gussakovsky, E.E., Gal, E., Ganelevin, R., 2004. Colormets: crop protection and light-quality manipulation in one technology. *Acta Hort.* 143–151. <https://doi.org/10.17660/ActaHortic.2004.659.17>.
- da Silva, M.A., Ferreira, N., da S., Teixeira-Souza, V.H., da S., Maia-Silva, C., de Oliveira, F., de A., Hrcir, M., 2017. On the thermal limits for the use of stingless bees as pollinators in commercial greenhouses. *J. Apic. Res.* 56, 81–90. <https://doi.org/10.1080/00218839.2016.1260380>.
- Snelgar, W.P., Manson, P.J., Hopkirk, G., 1991. Effect of overhead shading on fruit size and yield potential of kiwifruit (*Actinidia deliciosa*). *J. Hortic. Sci.* 66, 261–273. <https://doi.org/10.1080/00221589.1991.11516153>.
- Solomakhin, A., Blanke, M., 2010. The microclimate under coloured hailnets affects leaf and fruit temperature, leaf anatomy, vegetative and reproductive growth as well as fruit colouration in apple. *Ann. Appl. Biol.* 156, 121–136. <https://doi.org/10.1111/j.1744-7348.2009.00372.x>.
- Stamps, R.H., 2009. Use of Colored Shade Netting in Horticulture. *horts* 44, 239–241. <https://doi.org/10.21273/HORTSCI.44.2.239>.
- Stroka, M.A., Ayub, R.A., Silva, D.M., da, Pessenti, I.L., Pereira, A.B., Barbosa, E.A.A., 2021. Effect of anti-hail nets with different colors on 'Eva' apple trees agronomical responses. *e-157 Rev. Bras. Frutic.* 43. <https://doi.org/10.1590/0100-29452021157>.
- Taber, S.K., Olmstead, J.W., 2016. Impact of Cross- and Self-pollination on Fruit Set, Fruit Size, Seed Number, and Harvest Timing Among 13 Southern Highbush Blueberry Cultivars. *hortte* 26, 213–219. <https://doi.org/10.21273/HORTTECH.26.2.213>.
- Team, R.C., 2021. R: a language and environment for statistical computing. (Version 4.1.0)[Computer software]. Vienna: R Foundation for Statistical Computing.
- Tollington, S., Kareemun, Z., Augustin, A., Lallchand, K., Tatayah, V., Zimmermann, A., 2019. Quantifying the damage caused by fruit bats to backyard lychee trees in Mauritius and evaluating the benefits of protective netting. *PLoS ONE* 14, e0220955. <https://doi.org/10.1371/journal.pone.0220955>.
- Whitaker, K., Middleton, S., Whitaker, K., Middleton, S., 1999. Profitab. Hail Netting Apple Orchards. <https://doi.org/10.22004/AG.ECON.125036>.